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CHARACTERIZATION OF THE CELLULAR STRESS RESPONSE INDUCED BY EXPOSURE TO ORGANIC COMPUNDS

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13. ABSTRACT (Maximum 200 words)

Experiments were conducted for this project to determine if various characteristics of the cellular stress response among tissues could serve as the basis for developing a suite of biomarkers that: (1) identify vulnerable target tissues; (2) delineate the cellular manifestations of toxicity; and (3) screen for exposure to specific classes of organic contaminants. Relationships were examined, at the cellular level, between accumulation and localization of stress proteins (stress-70, chaperonin60, and ubiquitin) and alterations in subcellular compartments using organic compounds with well characterized proteotoxic and genotoxic effects. These objectives were accomplished using the following strategies: 1. Well characterized toxins were used to test the hypothesis that the induction and/or subcellular localization of stress-70, and chaperonin, and ubiquitin, reflects the intracellular sites of toxicity of compounds with established mechanisms of toxicity; 2. Induction and subcellular localization of stress-70, chaperonin, and ubiquitin by novel compounds were compared with the cellular manifestations of toxicity, 3. The pattern of accumulation of stress proteins, as a result of exposure to these novel compounds, was characterized; 4. Determined if the above compunds results in unique tissue

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and subcellular patterns of stress protein accumulation; 5. Determined is the tissue and subcellular patterns of stress protein accumulation could be used as "signatures" to distinguish compounds or classes of compounds from one another.

Development of a Suite of Biomarkers of Toxicity and Exposure Based on the Cellular Stress Response

Grant Number: 93-NL-119

Final Report

Principal Investigator: Brenda Sanders
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Objectives

Experiments were conducted for this project to determine if various characteristics of the cellular stress response among tissues could serve as the basis for developing a suite of biomarkers that:

(1) identify vulnerable target tissues;
(2) delineate the cellular manifestations of toxicity; and,
(3) screen for exposure to specific classes of organic contaminants.

Relationships were examined, at the cellular level, between accumulation and localization of stress proteins (stress-70, chaperonin60, and ubiquitin) and alterations in subcellular compartments using organic compounds with well characterized proteotoxic and genotoxic effects.

These objectives were accomplished using the following strategies:

1. Well characterized toxins were used to test the hypothesis that the induction and/or subcellular localization of stress-70, and chaperonin, and ubiquitin, reflects the intracellular sites of toxicity of compounds with established mechanisms of toxicity;

2. Induction and subcellular localization of stress-70, chaperonin, and ubiquitin by novel compounds were compared with the cellular manifestations of toxicity;

3. The pattern of accumulation of stress proteins, as a result of exposure to these novel compounds, was characterized;

4. Determined if the above compounds result in unique tissue and subcellular patterns of stress protein accumulation;

5. Determined if the tissue and subcellular patterns of stress protein accumulation could be used as "signatures" to distinguish compounds or classes of compounds from one another.

Experimental Protocols

Aquatic Model: *Poeciliopsis*

Heat shock and organic contaminant experiments were carried out over the course of this project on the hepatocyte cell line to characterize induction of the cellular stress response. Heat shock experiments were carried out to characterize the stress response in this species which is elicited in response to elevated temperatures and to compare these with data in the literature. Four families of stress proteins were analyzed (low molecular weight, hsp60, hsp70, hsp90) for the induction (radiolabeling experiments) and accumulation (western blotting) of stress proteins. These data were complimented with analyses of the level of protein damage using the antibody polyubiquitin which is a cellular tag of protein damage.

Heat Shock Treatments

Cells were cultured at 22, 27 and 32°C and grown to confluent monolayers prior to experiments. Cells were heat shocked in sealed T-75 flasks (n=3 or 4) in a circulating water bath at 6-16EC above ambient temperature for 30 minutes: 22°C cells were heat shocked at 32 and 38°C; 27°C cells were heat shocked at 38°C, and 32°C cells were heat shocked at 38° C and 40°C. They were returned to ambient temperature for 2 or 8 hours and harvested.

Benzo[a]pyrene Exposures

Cells were exposed to benzo[a]pyrene (in DMSO carrier) at 0.03-4 ug/ml B[a]P at 27EC for 24 hrs. Cell viability and visual health were noted at experiment termination. DMSO was not found to induce the hsp response at the concentration used (0.13% v/v). Experimental numbering system shown below indicates the fifth, sixth and seventh B[a]P exposures where B[a]P dissolved completely in the culture media.

DMSO (solvent carrier) Range-Finding Experiment

Cells were passed to 24-well plates and grown to confluence for 4 to 5 days at 27°C. Cells were exposed to media containing 0.005, 0.01, 0.05, 0.1, 0.25, 0.5 and 1% DMSO (v/v) for 24 hrs (n=9).

B[a]P V Exposure

Cells were passed to 24-well plates and grown to confluence for 4 to 5 days at 27EC. Cells were exposed to 0.015, 0.03, 0.06, 0.13, 0.25, 0.5, 1, 2 and 4 ug/ml (w/v) (or 0.06, 0.12, 0.25, 0.5, 1.0, 2.0, 4.0, 7.9 and 15.9 uM) B[a]P (containing 0.13% DMSO) for 24 hrs (n=9).

B[a]P VI Exposure

Cells resulting from 5 culture passes (6 weeks old) were passed to fresh 75 cm^2 flasks (n=10) and exposed to media containing B[a]P (0.03, 0.13, 0.25, 0.5, 1, 2 and 4 ug/ml (w/v) with 0.13% DMSO) for 24 hrs at 27EC.

B[a]P VII Exposure

Cells resulting from 30 culture passes (1 year old) were passed to fresh 75 cm^2 flasks (n=5) and exposed to media containing B[a]P (0.03, 0.13, 0.25, 0.5, 1, 2 and 4 ug/ml (w/v) with 0.13% DMSO) for 24 hrs at 27EC. Cell density approximated 1.8×10^5 cells/cm^2.

Terrestrial Model: *Earthworm*

Heat Shock Experiment I

Redworms were obtained from local bait shop and acclimated at 13EC. Worms were heat shocked in soil at 23EC for 1 hour and allowed to recover at ambient temperature overnight (17 hours).

Heat Shock Experiment II

Redworms were obtained from local bait shop and acclimated at 14EC. Worms were removed from soil, rinsed and placed on wetted filter paper in petri dishes (n=10). Worms were heat shocked at 28EC for 1 hour and allowed to recover at ambient temperature overnight (17 hours).

Heat Shock Experiment III

Redworms were obtained from local bait shop and acclimated at 17EC for 5 days. Worms were removed from soil, rinsed and placed on wetted filter paper in petri dishes (n=5). Worms were heat shocked at 25EC or 32EC for 30 minutes and allowed to recover at ambient temperature overnight (17 hours).

B[a]P Exposure I

Redworms were obtained from local bait shop and depurated in a cold room for 48 hours. Worms were placed on filter paper wetted with 0.25, 0.5, 1, 2, 4, 8 and 16 Φg/ml benzo[a]pyrene (in 0.08% N,N-dimethylformamide, DMF) for 10 days (n=5).

B[a]P Exposure II

Redworms were obtained from local bait shop and depurated in a cold room for 48 hours. Worms were placed on filter paper wetted with 0.25, 0.5, 1, 2, 4, 8 and 16 Φg/ml benzo[a]pyrene (in 0.08% N,N-dimethylformamide, DMF) for 10 days (n=12).

Methods

Metabolic Labeling

Metabolic labeling with ^{35}S-methionine/cysteine was used to trace the progress of cellular translation. Cells were heat shocked in sealed flasks in a circulating water bath at 6-16EC above ambient temperature for 30 minutes. They were then labeled with ^{35}S-methionine/cysteine (77 uCi/ml, trans-label ICN Research Products) for 2 hours at ambient temperature. Cells were collected by trypsinization, centrifuged at 600 x g for 3 minutes and homogenized.

Preparation of Proteins

Harvested cells were homogenized in 66.7 mM Tris, 0.1% nonidet and 0.1 mM phenylmethylsulfonylfluoride (PMSF) pH 7.5 and spun at 16,000 x g for 10 min at 4EC. The resulting supernatant was removed from the pellet and total protein was determined by the method of Bradford (1976) using Coomassie Brilliant Blue. Samples were frozen at -80°C until analysis.

Pellet Re-homogenization

Pellets were re-homogenized with a glass homogenizer on ice in the buffer described above plus 1.5% SDS (final concentration). This procedure dissolved protein aggregates remaining in the cell pellet. Homogenates were centrifuged at 16,000 x g for 10 min at 4EC, supernatants were removed from the pellets (if a pellet formed) and protein concentration was determined using the Bradford method (1976).

One-Dimensional Gel Electrophoresis

Protein samples were boiled for 8 min with sodium dodecyl sulfate (SDS) sample buffer (Laemmli 1970) at a ratio of 4 parts sample to 1 part Laemmli buffer. Equal total protein (5 Φg for mono70 and 40 Φg for poly60 for western blotting analyses; 30 Φg for metabolic labeling) for each sample was separated on 12.5% polyacrylamide gels with 5% stacking gels as described by Blattler et al. (1972) and electrophoresis was performed using the discontinuous buffer system of Laemmli (1970). Gels were run for 1 hr at 50 mAmps/gel. Gels were then either used for electroblotting or were stained for beta counter scanning.

Gels containing radiolabeled samples were stained with Coomassie blue, destained with 7.5% methanol:7.5% acetic acid, dried on a slab gel drier (Hoefer), scanned for quantification, and autoradiographed. Dried hot gels were scanned directly for 18 hrs using an AMBIS Beta Scanning System to determine cpm/Φg protein for each sample and exposed to Kodak X-AR film to produce autoradiographs.

Western Blotting

Western blotting was used to estimate the accumulation of hsp60 and hsp70 as well as estimate the levels of cellular protein damage. Samples separated by 1-D gel electrophoresis were electroblotted onto 0.45 Φm Immobilon-P polyvinylidene difluoride (PVDF) transfer membranes (Millipore), blocked in milk and probed with primary antibodies. A polyclonal antibody directed against moth (*Heliothis virescens*) hsp60 (Miller, 1987) and a monoclonal antibody directed against human hsp70 (clone 3a3, MA3-006, Affinity Bioreagents) were used as probes for the stress proteins hsp60 and hsp70. A polyclonal anti-ubiquitin antibody (U-5379, Sigma) was used to estimate the level of protein damage. Membranes probed for hsp60 and hsp70 were incubated with alkaline phosphatase detection reagents 5-bromo-4-chloro-3-indolyl phosphate p-toluidine salt (BCIP) and *p*-nitroblue tetrazolium chloride (NBT) and bands were scanned to obtain optical densities. Blots used for polyubiquitin analysis were developed using enhanced chemiluminescence (ECL) detection reagents. These blots were exposed to Kodak X-AR film and the bands were scanned for optical densities.

Data analysis

A mean and standard deviation were determined for all optical densities/ug and cpms/ug obtained for each treatment. Statistical analysis was run using SPSS for Windows version 3.1 at %=0.05. Levene's test for homogeneity of variances was performed prior to ANOVA. If variances were equal, parametric statistics were run using one way ANOVA followed by Student-Newman-Keuls, where appropriate. If variances were not equal, nonparametric statistics were run using the Kruskal-Wallis one-way ANOVA followed by Mann-Whitney U pairwise comparisons, where appropriate. The experiment-wise error rate was corrected for multiple pairwise comparisons between group means to control overall error rate using the Bonferroni Correction.

Results

Aquatic Model: *Poeciliopsis*

Cell line culture temperature appeared to modulate constitutive levels of hsps and determine the nature of the heat shock response (HSR) in this cell line. This cell line required more severe heat shock (HS) temperatures to induce more vigorous HSRs at higher culture temperatures. Hsp60 and hsp70 accumulation levels (as determined by western blotting methods) were highest at 27EC. Supernatant hsp60 levels were lowest at 32EC, suggesting that a significant proportion of hsp60 was in an "insoluble" pellet fraction. Hsp60 still showed a significant increase in accumulation at these low levels. Ubiquitination of cellular proteins increased with moderate HS yet decreased with more severe HS suggesting that the ubiquitin system is less important than the protective function of hsps at these temperatures. It appeared that constitutive levels of hsps vary with culture temperature, greater increases in culture and HS temperatures lead to more vigorous HSRs, and ubiquitination of cellular proteins is a coordinated and complementary system to the HSR during thermally challenging conditions.

In addition to heat shock experiments, four sets of experimental in year 2 and 3 in year 3 were performed with this cell line using the organic contaminant benzo[*a*]pyrene as the stressor (Figures 1-5). Analysis of the stress proteins hsp60 and hsp70 suggested that no significant induction of the stress response occurred following exposure to this contaminant. These results were quite puzzling. After communications with other researchers that have used these cells, it was determined that there are significant differences in the response between different clones of the culture. In addition other factors appear to modulate the response. During this series of experiments, a number of environmental factors were adjusted which we determined after the fact may affect the stress response: cell density, cell line age (number of passes), and photoactivation of polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene. Priliminary experiments were conducted to better understand the extent to which these factors contributed to variability.

A breeding population of *Poeciliopsis* was established in the laboratory and the goal was to obtain enough fishes for experiments at the organismal level to identify target tissues. The difficulty of obtaining adequate numbers of fishes, a high mortality and small size of individuals did not allow for these experiments to be conducted.

Terrestrial Model: *Earthworm*

After many attempts at optimizing the immunoblotting techniques for earthworms we were less than successful and decided to focus on the fish experiments. We did obtain data from a series of experiments conducted the previous year but they displayed at a very high level of variability which reduces our confidence in these data (fig. 6). After many unsuccessful attempts at optimizing the immunoblotting techniques for earthworms, further research after year 1 focused on the fish experiments.

Accomplishments/New Findings

Heat shock experiments characterizing the stress response in the fish cell line were performed and completed. Increases in culture temperature resulted in higher constitutive levels of hsp60 and hsp70 and stepwise increases in heat-shock treatments resulted in more vigorous induction of the heat shock response in this fish cell line.

This fish cell line did not appear to elicit a stress response to the organic contaminant benzo[*a*]pyrene under a broad range of environmental conditions. However it was determined that there are major differences in the stress response by different clones of this cell line. This complication was not clear when this model was used and these findings make it difficult to interpret some of the results obtained. In addition, the cells were very sensitive to such factors as number of passes before use in an experiment. Activation of the contaminant before exposing the fish may also be a major factor in determining the nature of the response. These findings make it difficult to interpret some of the results we have obtained.

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Bradford, M. B. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Laemmli, V. K. 1970. Cleavage of structural proteins during the assembly of the head bacteriophage T4. Nature 227: 680-685.

Personnel Supported

This research supported, in part:

Four Graduate Students:	Evan Moser, Anne Slaughter, Charles Moffett, Peggy Nakagawa
Two Undergraduates:	Taghi Vahdani-Sanavi, Reza Vahdani-Sanavi, Ruth Huang, Kim Chow, Tracy Yang
Two Laboratory Technicians:	Luis Cruz-Rodriguez, Chloe Taylor

Publications

1995 Di Giulio, R.T., W.B. Benson, B.M. Sanders and P.A. Van Veld. Biochemical

Mechanisms of Contaminant Metabolism, Adaptation and Toxicity. *In*: Fundamentals of Aquatic Toxicology. G. Rand (ed.) Taylor & Francis, Bristol, PA. p523-560.

1995 Sanders, B.M., J. Nguyen, L.S. Martin, S.R. Howe and S. Coventry. Characterization of the Cellular Stress Response Induced by Copper in the Fathead Minnow *Pimephales promelas*. Comp. Biochem. Physiol. C 112C(3):335-343.

1996 Sanders, B., P.L. Goering and K.D. Jenkins. The Role of General and Metal-Specific Cellular Responses in Protection and Repair of Metal-Induced Damage. *In*: The Toxicology of Metals. L. Chang (ed.) CRC Press, Inc., New York, NY. p165-187.

1996 Martin, L.S., S.R. Nieto and B.M. Sanders. Characterization of the Cellular Stress Response in Aquatic Organisms. *In*: Methods in Aquatic Toxicology. G. Ostrander (ed.) CRC Press, Inc., New York, NY. p341-369.

1996 Nakagawa, P.A., D.L. Soltz and B.M. Sanders. Characterization of the Cellular Stress Response in the Amargosa Pupfish, *Cyprinodon nevadensis (in review)*.

1997 Slaughter, A.M. 1997. The Heat Shock Response in the Top Minnow (*Poeciliopsis lucida*) PLHC-1 Cell Line. Masters Thesis, Humboldt State University, December.

Interactions/Transitions

a. Meetings, Conferences and Seminars

1994 B.M. Sanders and J. Nguyen. Acclimation alters the heat-inducible localization of cpn60 in the nucleus of a fish cell line. Heat Shock Proteins and Molecular Chaperones. Cold Spring Harbor, NY.

1994 P.A. Nakagawa, D.L. Soltz and B.M. Sanders. The cellular stress response and adaptation in the Amargosa pupfish, *Cyprinodon nevadensis*. American Society of Ichthyologists and Herpetologists Annual Meeting. Los Angeles, CA

1994 B.M. Sanders. Induction of the Cellular Stress Response as an Indicator of Damage. Society of Environmental Toxicology and Chemistry 25th Annual Meeting. Denver.

1994 University of Georgia, Savannah River Site, "An Environmental Perspective of

the Cellular Stress Response, March.

1994 Bodega Bay Marine Laboratory, UC Davis. The Cellular Stress Response: Implications for Environmental Physiology and Toxicology, November.

1995 Scripps Institute of Oceanography, UC San Diego. Stress Proteins in Aquatic Organisms, June.

1995 B.M. Sanders (invited). Stressor Specificity and the Cellular Stress Response: Implications for Adaptation, Environmental Monitoring and Toxicology. 4th International Congress of Comparative Physiology and Biochemistry, Birmingham, August.

1995 B.M. Sanders. The Use of Stress Proteins as Biomarkers of Contaminant specific Exposure. PICES Fourth Annual Meeting, Qingdoa, China, October.

1996 B.M. Sanders, H. Ellison and A. Slaughter. Stress Protein Levels in the Tissues of Bivalves Exposed to NAVSTA San Diego Sediments, Annual Meeting of the American Society of Limnology and Oceanography, San Diego, Feb.

1996 H. Ellison, S. Steinert, M. Salizar and B. Sanders. Induction of the Cellular Stress Response by *Mytilus edulis* upon exposure to PAHs and Metals in San Diego Bay. Southern California Academy of Sciences, Los Angeles, May.

1996 H. Ellison, S. Steinert and B. Sanders. Induction of the Cellular Stress Response by *Mytilus edulis* upon Contaminant Exposure *in situ*. 17th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Washington D.C., November.

1996 Anderson, J.W., K. Bothner, S. Steinert and B. Sanders. Induction of CYP1A1 in a Human Cell Line Exposed to Extracts of Mussels, as Measured by the P450 RGS Assay. 17th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Washington D.C., November.

b. Consultative and Advisory Functions: none

c. Transitions

My efforts with StressGen, a Canadian biotechnology company has continued. They are

interested in developing products that would apply many of the techniques that have been developed in our laboratory based upon expression of the stress response to routine screening of environmental samples. The data from this research and previous work in our laboratory contributes to the viability of the commercial application of these products. StressGen has licensed the rights for these from the University. For additional information contact Bette Kosmolak at StressGen (604-744-2811).

Patent Disclosures

1993 Sanders, B.M., K.D. Jenkins, J.L. Nichols and B.E. Imber. Accumulation of heat shock proteins for evaluating biological damage to chronic exposure of an organism to sublethal levels of pollutants. United States Patent Number 5,232,833. Date of Patent Aug. 3.

1995 Sanders, B.M., K.D. Jenkins, J.L. Nichols and B.E. Imber. Accumulation of heat shock proteins for evaluating biological damage to chronic exposure of an organism to sublethal levels of pollutants. Canadian Patent Number 1335558. Date of Patent May 16.

1995 Sanders, B.M., K.D. Jenkins, J.L. Nichols and B.E. Imber. Accumulation of heat shock proteins for evaluating biological damage to chronic exposure of an organism to sublethal levels of pollutants. United States Patent Number 5,464,750. Date of Patent Nov 7.

Honors/Awards

1996 Session Chair, Natural Resource Damage Assessments. 17th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Washington D.C., November.

1995-1998 Appointment to the Scientific Working Group of the North Pacific Marine Science Organization (PICES) to Practical Assessment Methodology (PAM).

1995-1997 Board of Directors, Society of Toxicology and Chemistry, Southern California Chapter.

1992-1995 Appointment to the Scientific Working Group of the North Pacific Marine Science Organization (PICES) to Develop Common Assessment

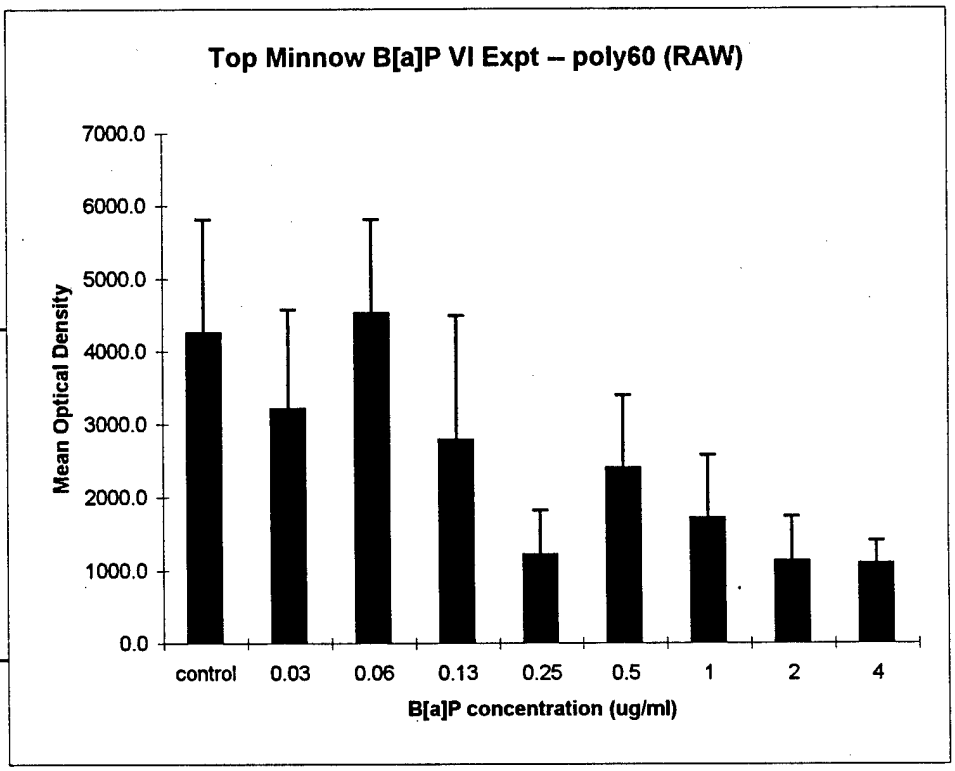
Methodology fo Marine Environmental Quality (MEQ).

1990-present	North American Editor, Ecotoxicology Series, Chapman and Hall Publishers, London.
1993	Distinguished Faculty Scholarly and Creative Achievement Award, California State University, Long Beach (lifetime award).

Top Minnow B[a]P Experiment VI

cpn60 Sample	Raw OD
C-1	7249.1
C-2	5355.3
C-3	3865.6
C-4	
C-5	3468.1
C-6	2680.0
C-7	4382.2
C-8	5532.3
C-9	2341.5
C-10	3406.4
0.03-1	3540.6
0.03-2	
0.03-3	1982.9
0.03-4	2476.8
0.03-5	2214.4
0.03-6	1832.1
0.03-7	6160.9
0.03-8	3091.4
0.03-9	4267.8
0.03-10	3333.5
0.06-1	5859.6
0.06-2	5501.9
0.06-3	'(7611.2)
0.06-4	4928.4
0.06-5	'(1659.3)
0.06-6	2990.1
0.06-7	3370.6
0.06-8	'(1713.9)
0.06-9	'(1433.3)
0.06-10	
0.13-1	5743.3
0.13-2	'(862.2)
0.13-3	1376.6
0.13-4	3493.3
0.13-5	'(791.7)
0.13-6	1758.5
0.13-7	
0.13-8	'(76.9)
0.13-9	3022.9
0.13-10	1283.6
0.25-1	
0.25-2	
0.25-3	
0.25-4	
0.25-5	769.5
0.25-6	
0.25-7	
0.25-8	1638.1
0.25-9	
0.25-10	
0.5-1	4180.7

ug/ml B[a]P	Raw OD values Mean	StDev	sd/mean	n
control	4253.4	1559.5	37%	9
0.03	3211.2	1364.7	42%	9
0.06	4530.1	1283.2	28%	5
0.13	2779.7	1708.8	61%	6
0.25	1203.8	614.2	51%	2
0.5	2391.7	998.7	42%	7
1	1702.0	864.1	51%	7
2	1116.4	609.9	55%	5
4	1086.5	312.8	29%	8



0.5-2	1247.7
0.5-3	2723.5
0.5-4	2540.7
0.5-5	'(819.9)
0.5-6	2457.7
0.5-7	1235.4
0.5-8	
0.5-9	2356.3
0.5-10	
1-1	3374.7
1-2	'(6146.2)
1-3	'(4369.1)
1-4	2286.1
1-5	1012.3
1-6	933.1
1-7	1557.3
1-8	1515.0
1-9	1235.2
1-10	
2-1	--
2-2	859.9
2-3	2039.5
2-4	789.9
2-5	1396.8
2-6	496.1
2-7	
2-8	'(129.5)
2-9	'(41.3)
4-1	'(5569.5)
4-2	791.9
4-3	1032.4
4-4	1042.0
4-5	922.1
4-6	856.5
4-7	1214.4
4-8	1789.9
4-9	'(4341.7)
4-10	1042.5

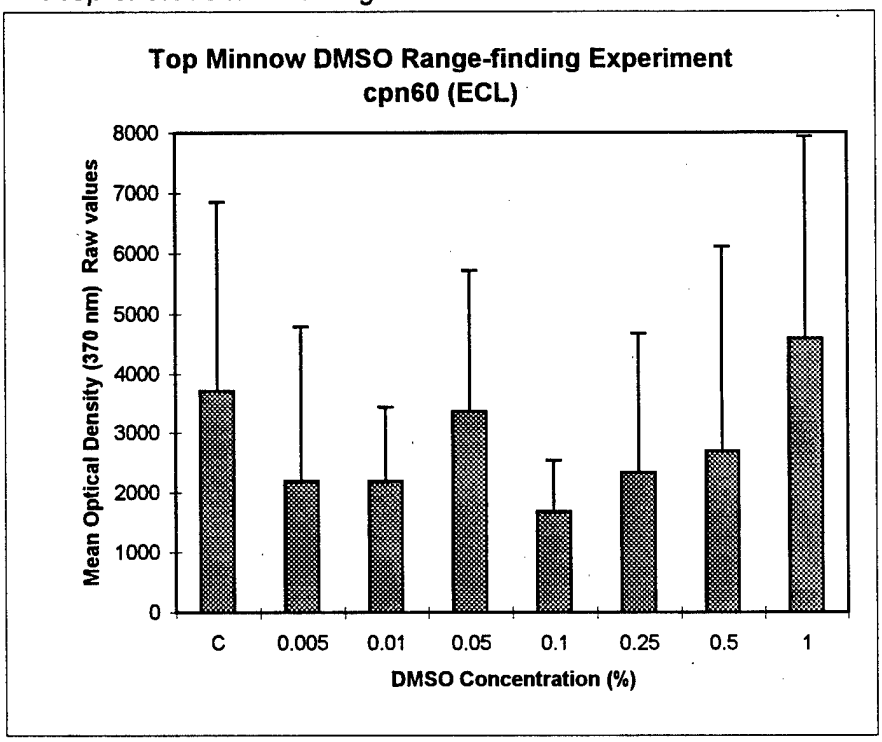
Top Minnow DMSO and BaP V Range-finding Experiments
Western optical densities (5/1/96 blots)

DMSO (Miller Poly60, 20 ug TP)

Blot #	Lane #	Sample ID	OD
5	2	NIST	1382.4
	3	1-1	7951.0
	4	0.1-5	3173.1
	5	0.005-5	1646.3
	6	0.5-1	99.6
	7	0.01-4	3366.4
	8	0.05-2	2130.6
	9	0.005-1	5959.1
6	2	NIST	86.3
	3	0.5-2	2424.7
	4	0.05-6	7128.0
	5	0.25-4	220.0
	6	1-2	2533.3
	7	0.01-3	1199.3
	8	0.1-1	1377.1
	9	0.5-4	2092.7
7	2	0.005-2	149.7
	3	0.5-3	324.9
	4	0.05-7	835.2
	5	0.25-3	2234.2
	6	0.1-4	1271.4
	7	1-3	8458.2
	8	0.01-1	3722.7
	9	NIST	--
8	3	0.1-2	1015.2
	4	1-4	1346.0
	5	0.05-1	3101.2
	6	0.01-5	1336.9
	7	0.25-2	5608.0
	8	0.005-3	18381.5 *
	9	NIST	1009.2
9	2	1-5	2609.1
	3	0.25-1	1274.4
	4	0.1-3	1573.8
	5	0.01-6	1345.6
	6	0.5-5	8505.2
	7	0.05-5	3574.7
	8	0.005-4	1017.2
	9	NIST	12.5

	Mean	StDev	StDev/Mean	n
NIST	622.6	679.8	109%	4
C	3711.5	3138.4	85%	3
0.005	2193.1	2584.6	118%	4
0.01	2194.2	1240.5	57%	5
0.05	3353.9	2355.7	70%	5
0.1	1682.1	857.5	51%	5
0.25	2334.2	2332.4	100%	4
0.5	2689.4	3411.3	127%	5
1	4579.5	3351.7	73%	5

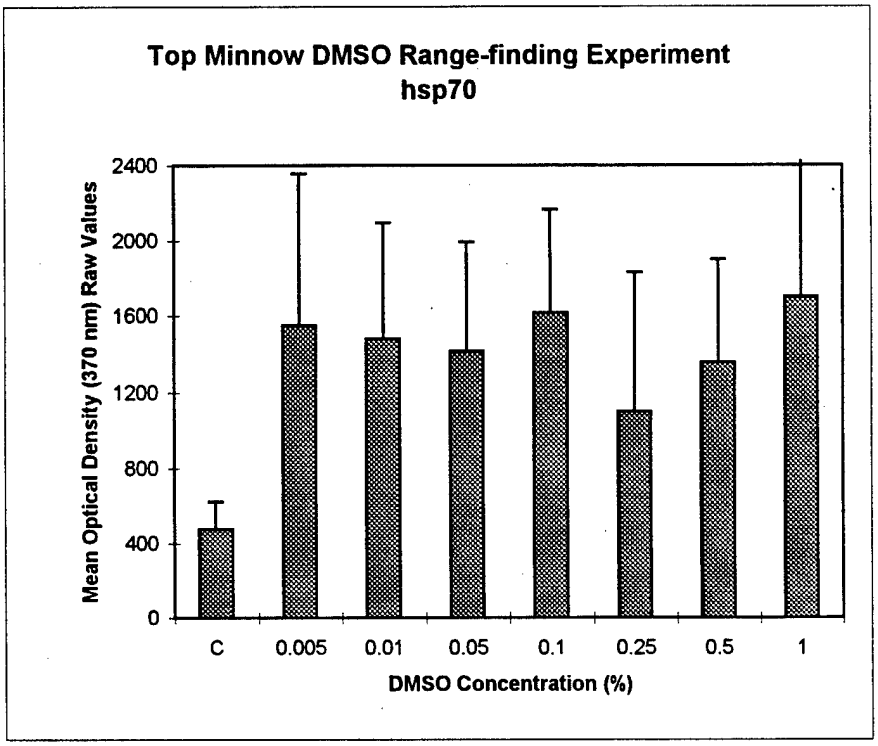
4 reps of 0.25 had < 20 ug TP
1 rep of 0.1 had <20 ug TP
1 rep of 0.005 had <20 ug TP



DMSO (3a3 Mono70, 20 ug TP)

Blot #	Lane #	Sample ID	OD
5	2	NIST	94.4
	3	1-1	1540.8
	4	0.1-5	1966.8
	5	0.005-5	1069.4
	6	0.5-1	959.1
	7	0.01-4	1018.6
	8	0.05-2	1400.3
	9	0.005-1	2159.4
6	2	NIST	23.4
	3	0.5-2	2146.4
	4	0.05-6	2337.0
	5	0.25-4	1684.5
	6	1-2	2941.8
	7	0.01-3	1908.9
	8	0.1-1	2121.5
	9	0.5-4	1585.9
7	2	0.005-2	2601.8
	3	0.5-3	771.7
	4	0.05-7	1330.3
	5	0.25-3	1761.3
	6	0.1-4	1937.5
	7	1-3	1948.6
	8	0.01-1	2288.3
	9	NIST	28.0
8	3	0.1-2	902.3
	4	1-4	667.4
	5	0.05-1	751.0
	6	0.01-5	819.2
	7	0.25-2	340.2
	8	0.005-3	640.6
	9	NIST	61.2
9	2	1-5	1388.4
	3	0.25-1	610.8
	4	0.1-3	1142.4
	5	0.01-6	1364.7
	6	0.5-5	1304.9
	7	0.05-5	1266.2
	8	0.005-4	1278.0
	9	NIST	110.1

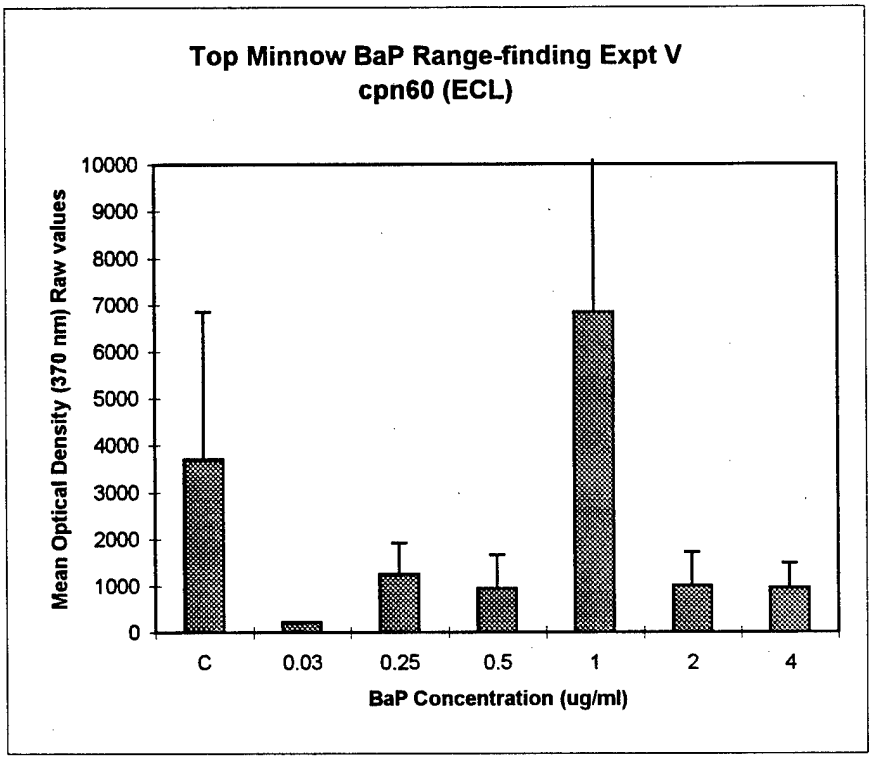
	Mean	StDev	StDev/Mean	n
NIST	63.4	38.7	61%	5
C	479.9	143.4	30%	3
0.005	1549.9	807.7	52%	5
0.01	1479.9	612.4	41%	5
0.05	1417.0	574.5	41%	5
0.1	1614.1	551.3	34%	5
0.25	1099.2	729.3	66%	4
0.5	1353.6	542.9	40%	5
1	1697.4	835.6	49%	5



Bap (Poly60)

Blot #	Lane #	Sample ID	OD
1	2	NIST	417.9
	3	C(V)-2	7317.5
	4	1-3	174.0
	5	0.5-5	786.3
	6	2-5	764.1
	7	2-1	1865.3
	8	0.25-6	1401.1
	9	4-4	799.7
2	2	NIST	884.9
	3	1-5	761.2
	4	0.25-1	2011.4
	5	0.5-1	238.6
	6	4-5	770.1
	7	1-1	31501.8
	8	2-2	177.5
	9	C(V)-8	2219.9
3	2	NIST	1010.3
	3	0.25-2	1389.7
	4	0.5-2	2151.0
	5	1-2	593.7
	6	4-1	1368.9
	7	4-2	1595.8
	8	2-3	523.1
	9	0.25-5	1300.0
4	2	NIST	546.8
	3	C(V)-7	1597.0
	4	0.5-3	778.5
	5	1-4	1162.4
	6	2-4	1615.2
	7	0.25-7	188.3
	8	0.5-4	760.6
	9	0.03-1	219.3
	10	4-3	255.7

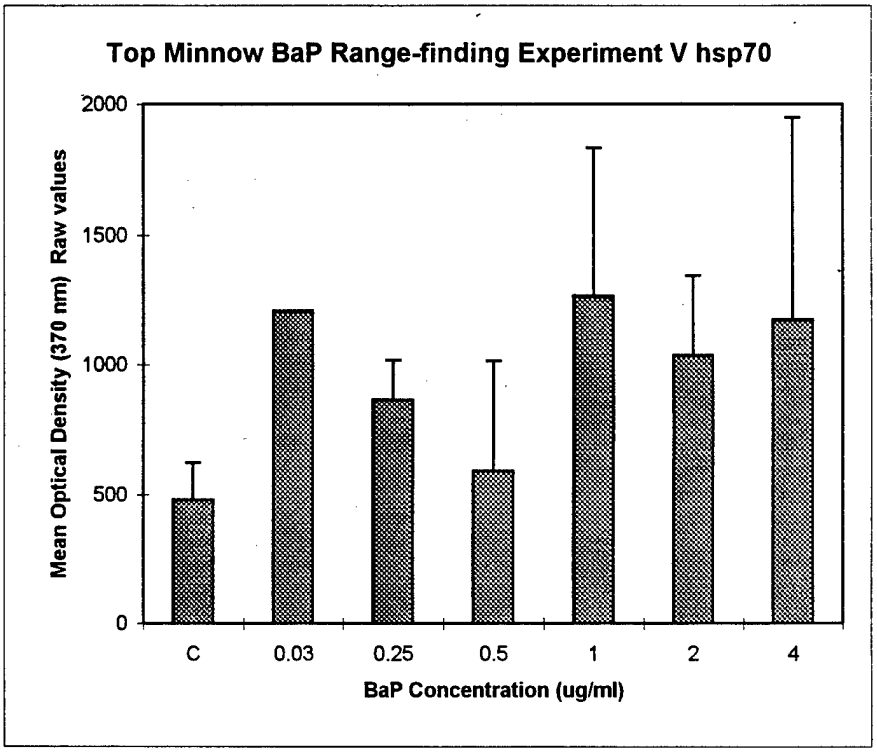
	Mean	StDev	StDev/Mean	n
NIST	714.9	278.5	39%	4
C	3711.5	3138.4	85%	3
0.03	219.3	--	--	1
0.25	1258.1	661.7	53%	5
0.5	943.0	714.2	76%	5
1	6838.6	13791.7	202%	5
2	989.0	722.2	73%	5
4	958.1	531.3	55%	5

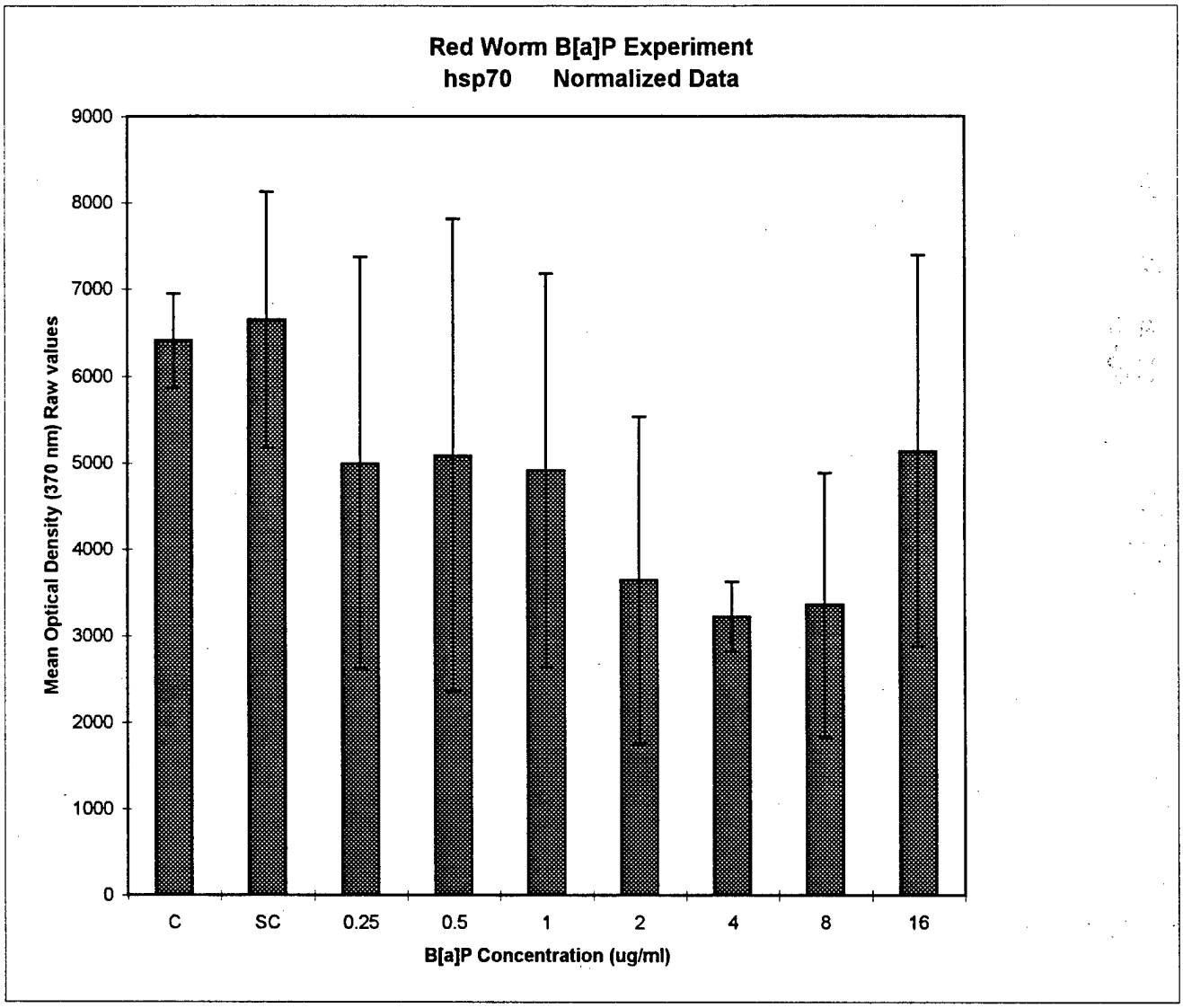


BaP (Mono70)

Blot #	Lane #	Sample ID	OD
1	2	NIST	54.7
	3	C(V)-2	362.0
	4	1-3	--
	5	0.5-5	64.9
	6	2-5	846.2
	7	2-1	1347.8
	8	0.25-6	666.6
	9	4-4	725.9
2	2	NIST	34.0
	3	1-5	738.9
	4	0.25-1	988.4
	5	0.5-1	1163.3
	6	4-5	556.4
	7	1-1	2059.6
	8	2-2	1059.7
	9	C(V)-8	438.3
3	2	NIST	80.7
	3	0.25-2	1042.8
	4	0.5-2	671.1
	5	1-2	1207.8
	6	4-1	2216.7
	7	4-2	1785.8
	8	2-3	1310.7
	9	0.25-5	772.8
4	2	NIST	38.8
	3	C(V)-7	639.6
	4	0.5-3	299.8
	5	1-4	1064.6
	6	2-4	619.2
	7	0.25-7	851.4
	8	0.5-4	755.7
	9	0.03-1	1207.9
	10	4-3	592.6

	Mean	StDev	StDev/Mean	n
NIST	52.1	21.1	40%	4
C	479.9	143.4	30%	3
0.03	1207.9	--	--	1
0.25	864.4	154.0	18%	5
0.5	591.0	425.0	72%	5
1	1267.7	563.2	44%	5
2	1036.7	309.4	30%	5
4	1175.5	771.7	66%	5




Red Worm B[a]P Experiment
hsp70 Normalized Data
Mean Optical Density (370 nm) Raw values
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
C
SC
0.25
0.5
1
2
4
8
16
B[a]P Concentration (ug/ml)